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COMPARATIVE ANATOMY OF THE TUNICATE TADPOLE, *CIONA INTESTINALIS*

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Abstract

At 19–20 h of age, the *Ciona intestinalis* ascidian tadpole has six developed organ systems (tunic, epidermis, notochord, tail musculature, adhesive organ, and nervous system) and approximately four organ system rudiments (including: primordial pharynx, atrial primordia, gut primordium, and mesodermal pockets). Contemporary light and electron microscopic descriptions of the anatomy and the histology of these tissues support the old contention, originated by Kowalevsky in 1866, that the body plans of tunicate tadpole and of vertebrate larvae are homologues.

Introduction

In the first volume of *Nature* (1869), there is a note entitled "Kinship of Ascidians and Vertebrates". It reads, in part:

> Prof. Kupffer . . . asserts that his [studies on the embryology of the tunicate *Phallusia*] in large measure agree with those of Kowalevsky touching the startling vertebrate features of the early condition of these invertebrata . . . He says: "At this stage one could not imagine a more beautiful model of a vertebrate embryo, with the neural tube on one side of the axis and a visceral tube on the other." . . . He promises full details shortly, and we hope to be able to return to this most important matter. (Foster, 1896)

Why were the vertebrate features of tunicate tadpoles called "startling"?

The tunicates were known to Aristotle: he called them *Tethya* or *Thalia*, and he was unsure whether they were plants or animals (Berrill, 1950; Singer, 1959; Barrington, 1965). Lamarck (1809), who named the group *Tunicata*, considered them to be molluscs. Cuvier (1828) classified the tunicates as shell-less acephalic molluscs; and likewise all other major biologists of the first half of the nineteenth century categorized tunicates as molluscs (Berrill, 1950). Thus, until the mid 1860s, the tunicates, a numerically significant group of organisms (Millar, 1971) with possibly 2000 extant species (Young, 1962), were thought to be prostomian invertebrates—exceedingly distant from the vertebrate phylogeny.

In 1866, this view changed dramatically when the comparative embryologist A. Kowalevsky discovered that the larval tunicate possesses a dorsal tubular nervous system, a ventral tubular gut, and an axial notochord flanked by muscles. In other words, the tunicate tadpole appears to have a characteristic vertebrate body plan. That the tunicates are actually members of the chordate phylogeny was irrefutably confirmed with the demonstrations by Kowalevsky (1866), Willey (1893a, b), and Conklin (1905) that the "mouth" (the 'pharynx') of the tunicate develops as a secondary opening at the end of the embryo farthest from the blastopore and that during embryogenesis the neural tube of the tunicate develops from the rolling up of a dorsal neural plate.

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Today, the tunicates are classified as urochordates, a subphylum of the chordates. This classification is based almost entirely on the larval anatomy (Berrill, 1950; Young, 1962; Barrington, 1965; Romer, 1970; Plough, 1978). Recent comparative biochemistry is consistent with this classification (Atkin and Ohno, 1967; Ohno, 1970; Schmidtke *et al.*, 1977, 1978; Fisher *et al.*, 1980). After their metamorphosis, most tunicates lose their notochords and their tubular nervous systems (Berrill, 1950; Cloney, 1977a), and the adult ascidian bears little resemblance to a contemporary chordate. ('Ascidian' is the class of sessile tunicates, commonly called the "sea squirts".)

Anatomical studies of the tunicate tadpole were pioneered by Kowalevsky (1866), Willey (1893a, b), Conklin (1905, 1931), Grave (1921), and Garstang (1928). Similar observations have also been reported by Scott (1946), Berrill (1947a, b), Abbott (1955), Trason (1957), and Anderson *et al.* (1976) (and summarized in: Herdman, 1932; Brien, 1948). The only anatomical studies using modern techniques have focussed on a few specific features, such as the sensory receptor cells (*e.g.*, Dilly, 1962, 1964; Barnes, 1971; Eakin and Kuda, 1971), the tail musculature (*e.g.*, Pucci-Minafra, 1965; Cavey and Cloney, 1972; Burighel *et al.*, 1977), and the notochord (Cloney, 1964, 1969). (For other examples, see: Mackie and Bone, 1976; Cloney, 1977b; Cloney and Cavey, 1982.) To interpret the old anatomical reports in the light of modern biological understanding, I have reexamined the anatomy of the tunicate tadpole using contemporary microscopic techniques. I have chosen to study the tadpole of the ascidian *Ciona intestinalis* because it is probably the most generalized of the readily available and well-studied tunicates (Berrill, 1950; Plough, 1978; J. R. Whittaker, personal communication).

Materials and Methods

Animals

Adult *Ciona intestinalis* were collected during June and July along the north shore of Cape Cod near Sandwich MA. The animals were maintained under constant light in running sea water at the Marine Biological Laboratory, Woods Hole. *Ciona* tadpoles were obtained by mixing sperm and eggs from three or four adults. The fertilized eggs were washed and then grown at 18°C in finger bowls of filtered sea water. All histology was done on newly hatched tadpoles, 19–20 h after fertilization. See Whittaker (1977) for a timetable of the developmental stages of *Ciona intestinalis* embryos raised at 18°C.

Histological techniques

Tunicate tadpoles were prepared for both light and electron microscopy using the same histological schedule, similar to the procedures described by Cloney and Florey (1968). All histological steps (except uranyl acetate staining) were done at room temperatures. First, the animals were fixed for 1 h in buffered 2.5% glutaraldehyde (2.5% glutaraldehyde in 0.215 *M* NaCl, 0.174 *M* Na_2HPO_4, 0.05 *M* sucrose, and 0.03 *M* NaH_2PO_4) and postfixed for ½ h in 1% osmium tetroxide. Washes were in a 1150 mOsm sodium phosphate buffer at pH 7.4–7.6. Next, tissues were dehydrated in ethanol, treated with propylene oxide, and then embedded in Epon 812. Thick sections (approx. one micron each) were stained with ½% toluidine blue for light microscopy, and thin sections were stained with 7.5% uranyl acetate (aqueous) for 10 min at 60°C followed by Reynold's lead citrate for 2 min at room temperature. Thin sections were examined at 80kV with a Zeiss EM 10CA transmission electron microscope.

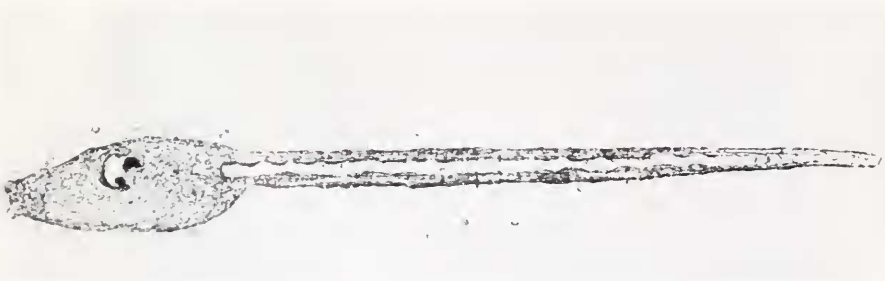

FIGURE 1. Light microscopic photograph of 19–20 h *Ciona intestinalis* tadpole. The outer tunic is covered by adherent inner follicle cells ('test cells'). The tadpole is quite transparent, and the two CNS pigment cells, the dorsal and the ventral melanocytes, can clearly be seen in the ventricle of the prosencephalon. The vacuolated notochord runs prominently down the center of the entire tail. (Photograph courtesy of T. H. Meedel and J. R. Whittaker, Boston University Marine Program, M.B.L., Woods Hole.)

RESULTS

The newly hatched tunicate tadpole is a tiny organism resembling a mammalian spermatozoan. At 19–20 h, *Ciona* tadpoles are uniformly 1.1 mm in length. The body is a tapering cylinder 0.2 mm long and 0.1 mm wide (Fig. 1). The tail is about 0.8 mm long and is 0.03 mm high and 0.02 mm wide at its largest cross-section. The tadpoles are quite transparent, and in living unstained organisms the most striking features are the two black pigment cells, the ventral and the dorsal melanocytes, of the prosencephalon of the central nervous system. With DIC (Nomarski) microscopy, most of the cells of the living tadpole can be distinguished at about 400× magnification. *Ciona* tadpoles swim in fits and starts from the time of hatching, but they do not feed. The earliest metamorphic changes can be detected about 36 h after fertilization.

The tissues of the tunicate tadpole can be usefully divided into two categories: the six developed organ systems (the "larval action-systems" [Grave, 1944; Scott, 1946]), and the four organ system rudiments.

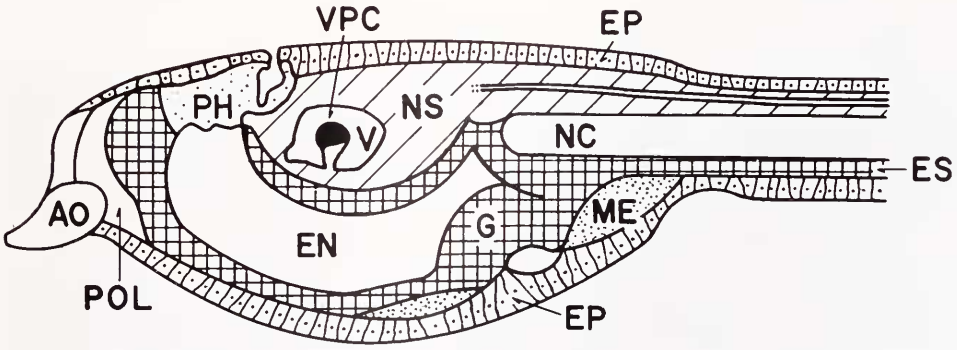

FIGURE 2. Mid sagittal section of 19–20 h *Ciona* tadpole, reconstructed with camera lucida from greater than 100 serial 1 μm plastic cross-sections. AO = adhesive organ, EN = endodermal cavity, EP = epidermis, ES = endodermal strand, G = gut primordium, ME = mesodermal pocket, NC = notochord, NS = nervous system, PH = primordial pharynx, POL = preoral lobe, V = ventricle of prosencephalon, VPC = ventral melanocyte.

FIGURE 3. Typical mitochondrion of tunicate tadpole (tail musculature, bar = 0.5 μm).

General histology

Cells in the tunicate tadpole tend to be small, less than 10 μm in diameter. The notable exceptions are the muscle cells of the tail which are mononucleated fusiform cells greater than 100 μm in length. All cells of the 19–20 h tadpole contain yolk vesicles, and the highest concentration of yolk vesicles is found in cells of the gut primordium. Yolk vesicles are bounded by a membrane, and internally they appear to be finely granular and homogeneous. Yolk vesicles are roughly spherical and range from about 1.5–3.0 μm in diameter. In general, mitochondria appear to be elliptical or dumb-bell shaped. They range in cross-sectional diameter from 0.4–0.6 μm and in length from 0.6–1.4 μm. All mitochondria have strikingly rectangular cristae and contain dense granules (Fig. 3). Throughout the tadpole, all nuclei appear to have prominent nucleoli. In the following sections, I describe vacuoles in certain cell types—such vacuoles can be the result of poor oxygenation of the animals prior to fixation (B. Tandler, personal communication), and it is possible that they may have been artificially exaggerated in my preparations.

Developed organ systems

1. Tunic

The tunicate tadpole is completely covered by an extracellular "skin" or tunic ('T' in Figs. 4–9) which forms the fins of the animal. The fins all run along the long axis of the animal. The largest fins consist of a dorsal and a ventral flap of tunic running the entire length of the tadpole. One or two major fins also run along each lateral surface of the body. In addition, many tiny fins and short finger-like projections are found along the outer surface of the tunic (Fig. 8).

The tunic itself is acellular. Its outermost edge is formed by a rind (the 'outer cuticle', Figs. 5–7) which is about 40–50 nm thick and which stains densely with a wide variety of EM and LM stains. The innermost edge forms another thinner (10–12 nm thick) rind, the 'inner cuticle' (Fig. 5). A number of highly vacuolated inner follicle cells (also called 'test cells') from the oocyte still adhere to the outer surface of the tadpole tunic (Fig. 1). Along the inner surface of the tunic, the epidermal cells form a generally smooth boundary, and cell processes are rarely found

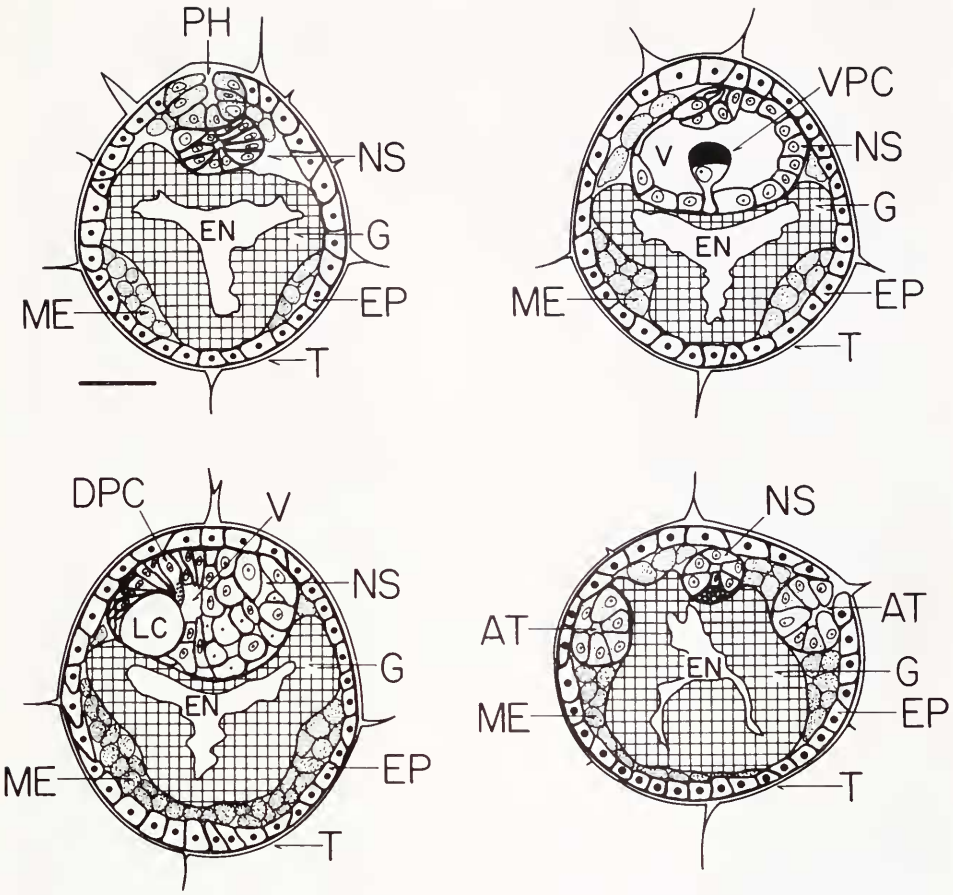

FIGURE 4. Camera lucida tracings of four 1 μm plastic cross-sections through the body of a 19–20 h *Ciona* tadpole. Proceeding rostrally to caudally: upper left = level of primoridial pharynx, upper right = level of ventral melanocyte, lower left = level of ocellus, lower right = level of atria. AT = atrial primordium, DPC = dorsal melanocyte, EN = endodermal cavity, EP = epidermis, G = gut primordium, ME = mesodermal pocket, NS = nervous system, PH = primordial pharynx, T = tunic, V = ventricle of prosencephalon, VPC = ventral melanocyte. Bar = 10 μm.

protruding into the tunic. There are, however, occasional epidermal cilia. (See description of epidermis, below, for more details.) Recent ultrastructural studies have examined the tunic and its development in detail and have described an irregular meshwork of filaments throughout the tunic (Dilly, 1969a; Mancuso, 1973, 1974; Gianguzza and Dolcemascolo, 1980; Cloney and Cavey, 1982).

2. Epidermis

A single-layered epidermis covers the entire animal (Figs. 2, 4–9, 11) except where the pharynx and the two atria break through. The combination of a single-layered epidermis and an outer tunic-like secretion (probably derived from the epidermal cells [Gianguzza and Dolcemascolo, 1980]) is more characteristic of invertebrate integument than of vertebrate integument (Spearman, 1973—see also Wellings and Brown, 1969). In the tunicate tadpole, the epidermal cells form a simple

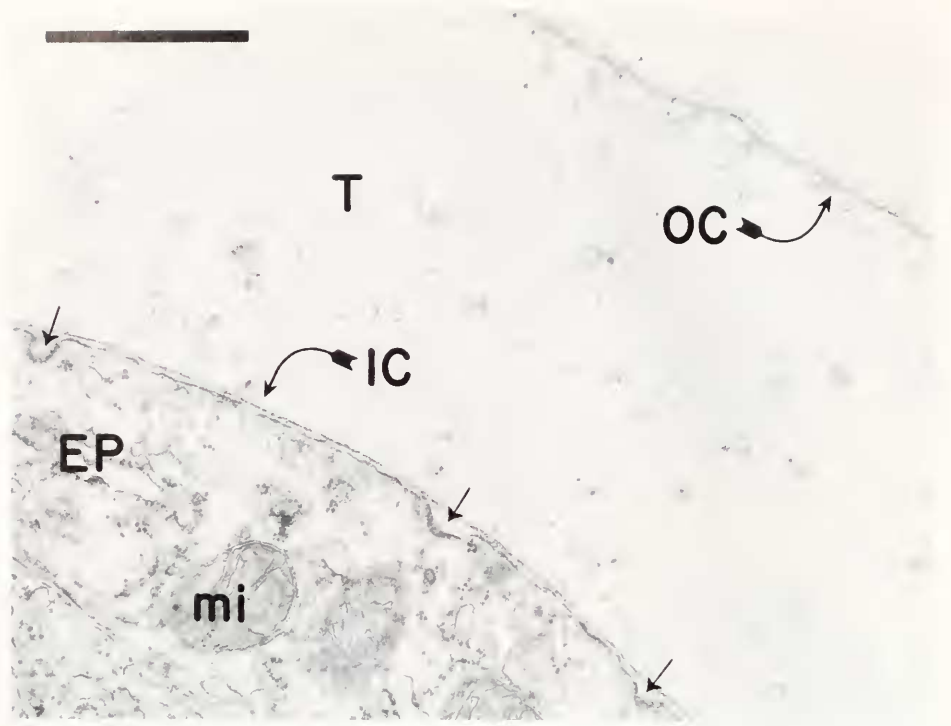

FIGURE 5. Electron micrograph of outer edge of epidermis in the tadpole body. Arrows point to coated vesicles in various stages of formation. EP = epidermal cell, IC = inner cuticle, mi = mitochondrion, OC = outer cuticle, T = tunic. Bar = 1 μm.

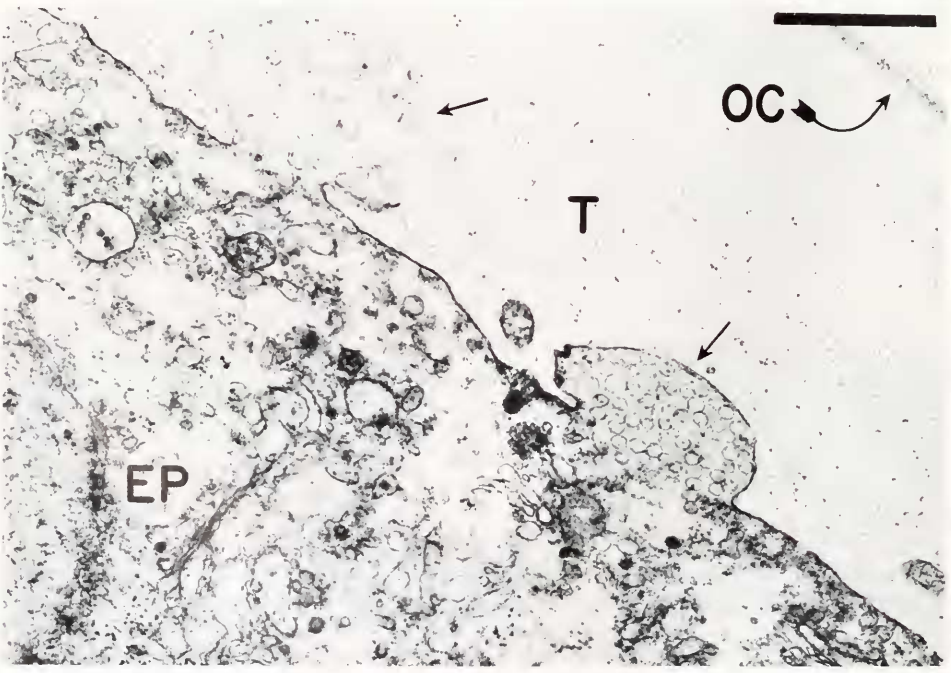

FIGURE 6. Electron micrograph of outer edge of epidermis in the tadpole tail. Arrows point to vesicles in two stages of exocytosis. EP = epidermal cell, OC = outer cuticle, T = tunic. Bar = 1 μm.

squamous or cuboidal epithelium. The thickest cells are about 9 μm from base to apex; these are found both dorsally and ventrally in the tail. At its thinnest (along the lateral walls of the tail), the epidermis is made of flattened cell processes only about 1 μm thick (Figs. 7, 8).

Throughout the epidermis, the outer cell surface is generally smooth without microvilli or extensive ridges. Notable exceptions are finger-like non-ciliated cell processes that extend as far as 2.5 μm into some of the longer fins of the tunic. In addition, as Dilly (1969a) has reported, occasional cilia (with a 9 + 2 microtubule arrangement) protrude from the outer edge of some epidermal cells. The external surface of the epidermis appears quite active, being studded with coated pits in all stages of formation (Fig. 5), especially along the body epidermis. The external surface also contains exocytotic vesicles, especially along the tail epidermis (Fig. 6). The boundaries between adjacent epidermal cells tend to be fairly smooth, although cells do interdigitate to some extent with their neighbors. Mackie and Bone (1976) and Georges (1979) have described gap and tight junctions but no septate junctions in the epidermises of a variety of tunicate tadpoles. Dilly (1969a) suggested the possibility of some desmosomes in the *Ciona* tadpole epidermis. (See Green and Bergquist [1982] for a review of the phylogeny of intercellular junctions.) The inner surfaces of the epidermal cells are fairly smooth and sit upon a basal lamina, which ranges in thickness from 40–120 nm. There is no underlying dermis.

The epidermal cells have large oval nuclei (3 × 4 μm in diameter). Some cross-sections show two nucleoli per nucleus, and this may be the standard complement for epidermal cells. Occasionally, the cells contain large (2–4 μm in diameter) vacuoles. The epidermal cells are not filled with special non-yolk vesicles, as they are in some related animals (*e.g.*, *Amphioxus* [Olsson, 1961]).

3. Notochord

Chordate larvae are characterized by a prominent notochord, which in anamniotes can be larger in diameter than the spinal cord (Goodrich, 1930; Neal and Rand, 1936; Leeson and Leeson, 1958; Bruns and Gross, 1970; Ruggeri, 1972; Jurand, 1974). In *Ciona* tadpoles, the notochord is a rod-shaped tissue, approximately 0.7 mm long and 9–10 μm in diameter, running the length of the tail and extending into the caudal body (Figs. 2, 7, 8, 10, 14). Dorsally, the notochord is covered by the deuterencephalon of the nervous system; ventrally, it overlies the endodermal strand; and laterally, it is surrounded by the muscles of the tail. The development of the notochord of *Ciona* has been well described by Conklin (1905), Cloney (1964), and Mancuso and Dolcemascolo (1977).

The notochord is composed of about 40 cells aligned longitudinally in single file. By the time of hatching, the notochord cells have become a simple squamous epithelium surrounding a matrix-filled lumen, with much the same geometry as a vertebrate endothelial capillary (Fig. 8). The anterior end of the notochord is capped by a single notochord cell. The basal surfaces of the notochord cells rest on a basal lamina that completely surrounds the notochord and that is termed the 'notochordal sheath'. The notochordal sheath ranges from 60–175 nm in thickness. It contains many circumferentially oriented and longitudinally oriented filaments (Cloney, 1964, 1969)—these extracellular fibers are arranged orthogonally, as is the case for certain vertebrate notochordal sheaths (*e.g.*, *Xenopus* tadpoles, M. J. Katz, unpublished observations). (For descriptions of the notochordal sheaths of other vertebrates, see: Leeson and Leeson, 1958; Jurand, 1962, 1974; Waddington and Perry, 1962; Bruns and Gross, 1970. Mathews [1967] provides a review of the evolution of connective tissue macromolecules, such as those forming the notochordal sheaths.)

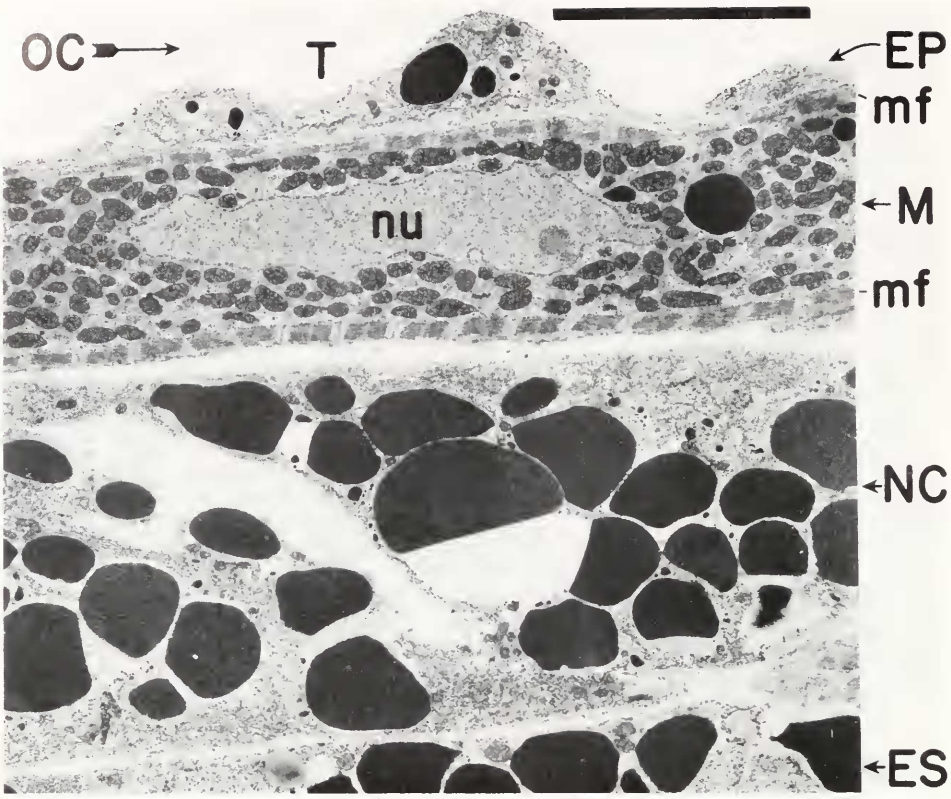

FIGURE 7. Electron micrograph of oblique longitudinal section through the tail. EP = epidermis, ES = endodermal strand, M = muscle cell, mf = myofibrils, NC = notochord, nu = nucleus of muscle cell, OC = outer cuticle, T = tunic. Bar = 1 μm.

The notochord cells themselves have irregularly shaped nuclei, which are about 4 μm in diameter. The cytoplasm is dark and quite granular, and the cells contain much moderately dilated rough endoplasmic reticulum (RER). The notochord cells also contain prominent yolk vesicles. More numerous than the yolk vesicles are other slightly larger (2.5–4 μm in diameter) membrane-bound vesicles that are filled with less densely packed material (Fig. 14). Occasionally, these vesicles can be seen budding off from the cell surface and appearing to empty their contents into the notochordal lumen ('star' in Fig. 8). In the notochordal cells, mitochondria are much more sparse than in the surrounding muscle and nervous system cells. The notochord cells of *Ciona* do not contain the thick bundles of intracellular filaments that are found in the notochord cells of *Amphioxus* (Eakin and Westfall, 1962; Flood, 1975b).

4. Tail musculature

The tail musculature is the most well studied of all the developed organ systems of tunicate tadpoles (*e.g.*, Berrill and Sheldon, 1964; Terakado, 1972; Ohmori and Sasaki, 1977; Whittaker *et al.*, 1977; Meedel and Whittaker, 1979; Whittaker, 1979a), and there are already several excellent ultrastructural reports (Pucci-Minafra, 1965; Castellani *et al.*, 1972; Cavey and Cloney, 1972, 1974, 1976; Bone *et al.*, 1977;

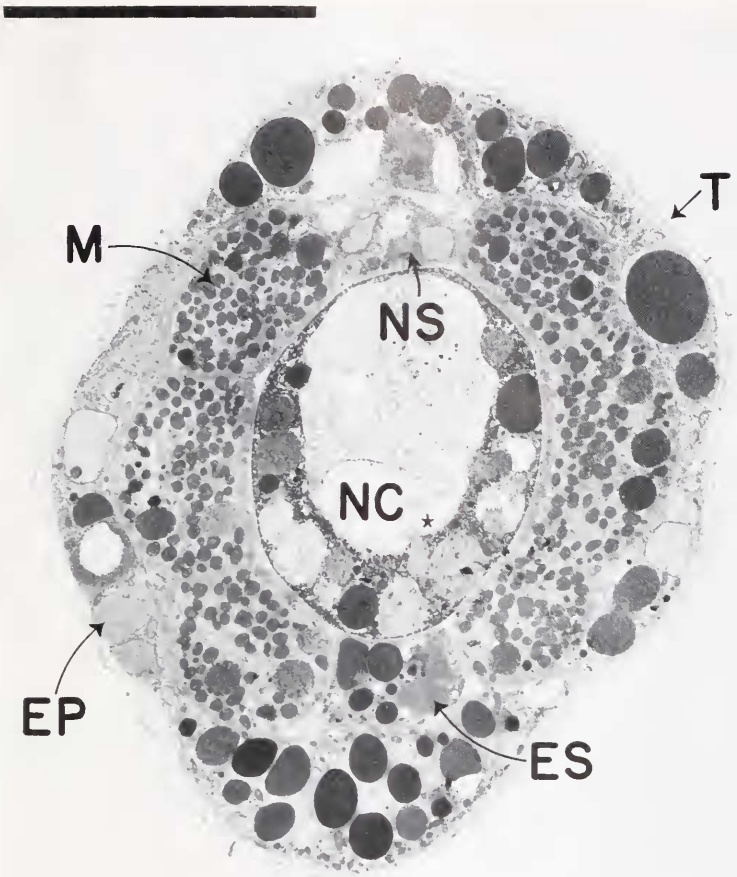

FIGURE 8. Electron micrograph of cross-section of tunicate tadpole tail. Star"' indicates apparent exocytosis of granular material into notochordal lumen. EP = epidermis, ES = endodermal strand, M = muscle cell, NC = notochord, NS = deuterencephalon of the nervous system, T = tunic. Bar = 10 μm. (Dorsal is at the top.)

Burighel *et al.*, 1977; Cavey, 1980). Therefore, I will only briefly describe the main features of the tail muscle system.

In *Ciona* tadpoles, the tail musculature comprises two sets of striated muscle cells. Each set of muscle cells is aligned as three longitudinal bands flanking the notochord laterally (Figs. 8, 14). Individual muscle cells are fusiform longitudinally and bean-shaped in cross-section. The dorsal and the ventral muscle cells are about 6 × 7.5 μm in cross-sectional diameter, and the middle muscle cells are about 6.5 × 10 μm in cross-sectional diameter. Muscle cells appear to be mononucleated; the nuclei are large (up to 5.5 μm in cross-sectional diameter and 15 μm long), irregularly shaped, centrally located, and contain a number of nucleoli (Fig. 7). The cytoplasm surrounding the nucleus is packed with mitochondria.

The myofibrils are all arranged in a single ring in the peripheral cytoplasm of the cell (Figs. 7, 8, 14). Myofibrils show the general vertebrate banding pattern

(Burighel *et al.*, 1977). Each muscle cell contains 20–35 myofibrils (each is 0.35 × 0.6 μm in cross-sectional diameter), and all the myofibrils are aligned in parallel, slightly oblique to the long axis of the muscle cell, *i.e.*, the long axis of the tail (Pucci-Minafra, 1965). There is no system of T-tubules. (T systems appear to be found only in tadpoles of those tunicate species with more centrally located myofibrils [Bone and Ryan, 1975; Cavey and Cloney, 1976; Burighel *et al.*, 1977]). Sarcoplasmic reticulum tubules completely surround each of the myofibrils. Between each Z band and the adjacent cell membrane there is a flattened and wider tubule or cisterna—these widened cisternae represent probable sites of electro-chemical coupling between cell membrane and myofibril (Burighel *et al.*, 1977).

Neuromuscular transmission is cholinergic (Mendes and Zingales, 1972; Ohmori and Sasaki, 1977; see also: Meedel and Whittaker, 1979), as is apparently the case for all complex metazoans except the arthropods (Mendes *et al.*, 1970; Gerschenfeld, 1973; Krnjevic, 1974; Rovainen, 1979).

5. Adhesive organ

Beginning at metamorphosis, the adhesive organ serves to attach the tunicate to a settlement surface (Cloney, 1977a). An adhesive organ consists of three cone-shaped protrusions—called 'papillae'—at the anterior end of the tadpole (Figs. 1, 2). The papillae of *Ciona* have a triangular arrangement, with one lying ventrally and two lying dorsally. In the 19–20 h tadpole, each papilla extends about 6 μm from the anterior end of the animal.

The papilla is a mass of packed cuboidal or spherical cells, each about 5 μm in diameter (Fig. 9). The cells are filled with quite dilated RER; they have few yolk vesicles and moderate-sized nuclei (3 μm in diameter). These cells resemble the 'collocytes' described by Cloney (1977b) for another suborder of ascidian. Except at their anterior tips, the papillae are enveloped by a single fairly uniform layer of flattened epidermal cells. Cloney (1977b) provides a detailed ultrastructural description of the papillae of a more complex ascidian larva, *Distaplia occidentalis*.

6. Nervous system

From its very beginnings, the central nervous system (CNS) of the tunicate tadpole is unlike that of any invertebrate. Most notably, the tunicate nervous system forms dorsally from a classic chordate neural plate, which then rolls into a neural tube (Kowalevsky, 1866; Conklin, 1905; Satoh, 1978). This neural tube establishes the dorsal tubular CNS of the tadpole.

In the 19–20 h *Ciona* tadpole, the CNS extends most of the length of the animal. Rostrally, it abuts the pharyngeal-gut junction, protruding under the primordial pharynx, *i.e.*, the incurrent siphon rudiment. Caudally, it reaches the end of the tail. The entire CNS is surrounded by a basal lamina averaging 150–250 nm in thickness. At 19–20 h, the CNS lumen is greatly expanded rostrally (to a maximum diameter of about 35 μm), is closed off entirely near the caudal end of the tadpole trunk, and is opened with a small uniform bore (about 1.5 μm in diameter) throughout the tail. By a number of anatomical criteria, the CNS can be divided into two parts: a *prosencephalon* rostrally and a *deuterencephalon* caudally (Fig. 10). (Kuhlenbeck [1975, 1977] provides an extensive review of the vertebrate prosencephalon and deuterencephalon.)

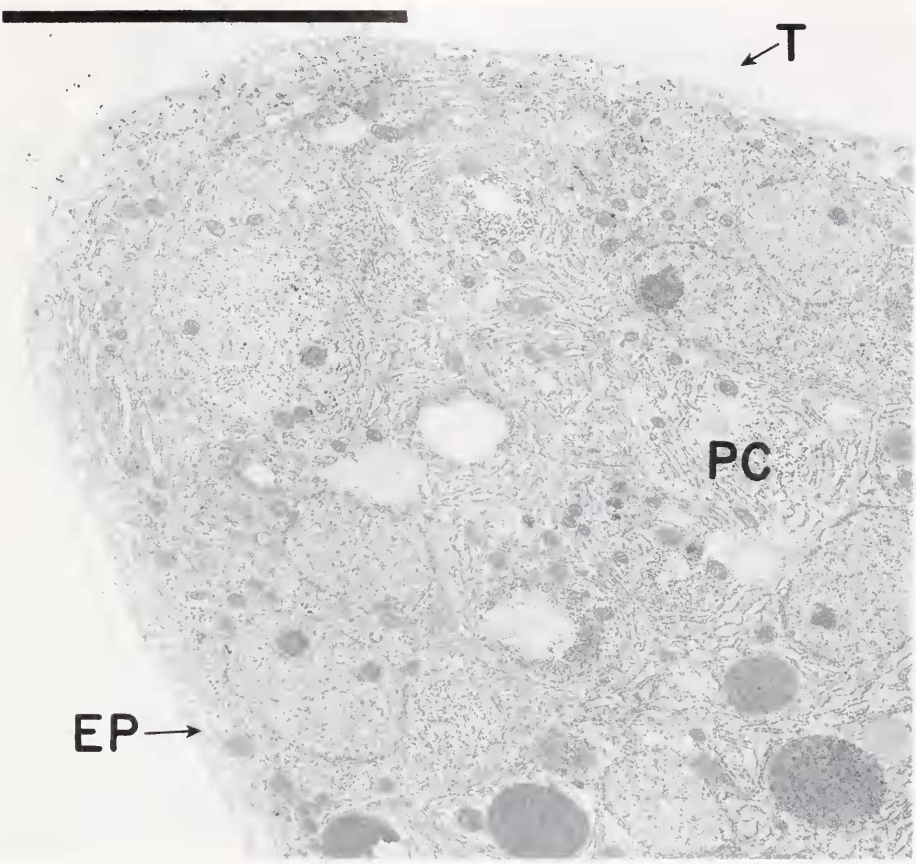

FIGURE 9. Electron micrograph of longitudinal section through one papilla of the adhesive organ. EP = epidermis, PC = papillar cell, T = tunic. Bar = 10 μm.

a. Prosencephalon

The prosencephalon is the bulbous anterior end of the CNS. It is located over the endodermal cavity of the gut primordium. Throughout its length, the prosencephalon tends to be only one cell thick. The prosencephalon contains a single large ventricle, along the inner walls of which lie two pigment cells. Ventrally in the midline, the ventral melanocyte ('VPC' in Figs. 2, 4, 11) sits like a golf ball and tee in the center of the ventricle. The ventral melanocyte has long been thought to be a sensory receptor (Kowalevsky, 1866; Dilly, 1962; Eakin and Kuda, 1971), and it is often called the 'otolith'. Its pigment—melanin (Whittaker, 1966, 1973, 1979b)—is collected intracellularly in a single hemispherical vesicle (with a radius of about 6 μm) capping the cell dorsally. The cell itself is round (13 μm in diameter) with a short ventral neck. The nucleus (4 μm in diameter) has slightly irregular contours, and the cytoplasm contains a number of yolk vesicles. The ventral melanocyte gives rise to an axon that passes caudally along the ventral rim of the prosencephalon and then into the deuterencephalon.

More caudally and dorsolaterally and to the right of the midline, the dorsal melanocyte ('DPG' in Fig. 11) lies embedded in the wall of the prosencephalon. The

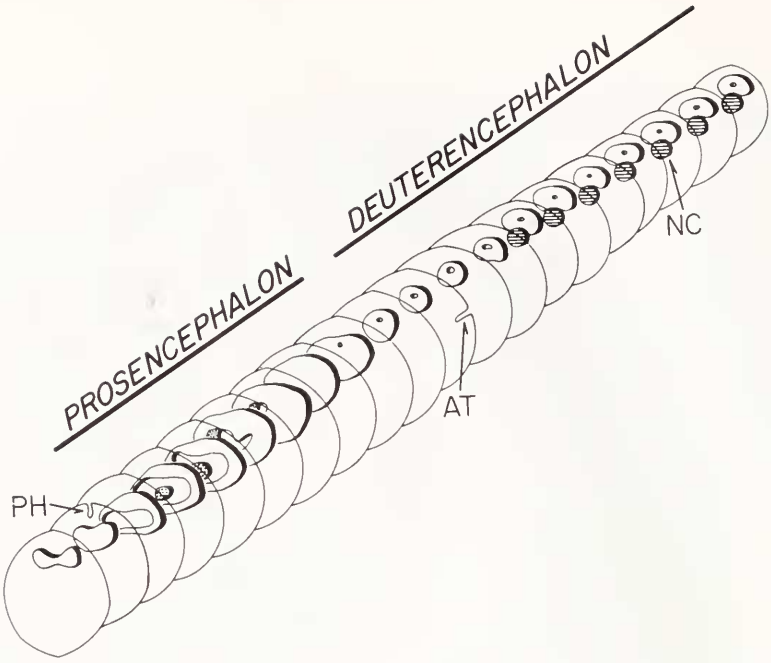

FIGURE 10. Series of sagittal camera lucida tracings through the CNS of the tadpole body (left to right is rostral to caudal). The prosencephalon of the CNS runs from beneath the primordial pharynx to just past the middle of the body. After a brief transitional zone, the deuterencephalon begins (at about the level of the atrial primordia) and runs along the dorsum of the notochord through the tail. The dorsal and the ventral melanocytes can be seen (dotted figures) in the ventricle of the prosencephalon. AT = atrial primordium, PH = primordial pharynx, NC = notochord.

dorsal melanocyte is part of the photoreceptor organ or 'ocellus' (Eakin, 1973). In this pigment cell, the melanin is found in a number of small (0.2–0.5 μm diameter), irregularly shaped vesicles distributed evenly throughout the apical cytoplasm. The dorsal melanocyte is columnar, with a length of 10–12 μm and a width of 3–4 μm in the wall of the prosencephalon. The luminal (apical) end of the cell then extends ventrally another 11–12 μm through the ventricle across the surfaces of the ventrally adjacent photoreceptor cells. The ciliary ends of these photoreceptors protrude 2.5–3.5 μm into the ventricle. The basal end of the pigment cell reaches the basal lamina of the CNS. The cytoplasm of the dorsal melanocyte is slightly paler than the cytoplasm of the adjacent photoreceptor cells, and its nucleus has an irregular outline, with a maximum diameter of about 4 μm.

After traversing the surfaces of the photoreceptor cells, the dorsal melanocyte makes contact with luminal filopodia of the largest cells in the CNS, the lens cells (Fig. 11). The *Ciona* tadpole has three lens cells (Eakin and Kuda, 1971). The lens cells are generally spherical with a diameter of about 18 μm, and they lie side-by-side in the right lateral wall of the prosencephalon. Their peripheral cytoplasm has a dense finely granular texture and contains many mitochondria. These cells contain large (up to 10 μm in diameter) spherical regions of cytoplasm that are free of organelles and that are filled with a finely granular material, more densely concentrated in the center of the regions. These large homogeneous regions are ringed by mitochondria and stain metachromatically with toluidine blue. Detailed ultrastructural descriptions of the dorsal melanocyte, the ciliary photoreceptor cells, and the lens cells of *Ciona intestinalis* tadpoles have been reported by Dilly (1964), Eakin and Kuda (1971), and Eakin (1973). (See also Barnes [1971, 1974] for similar de-

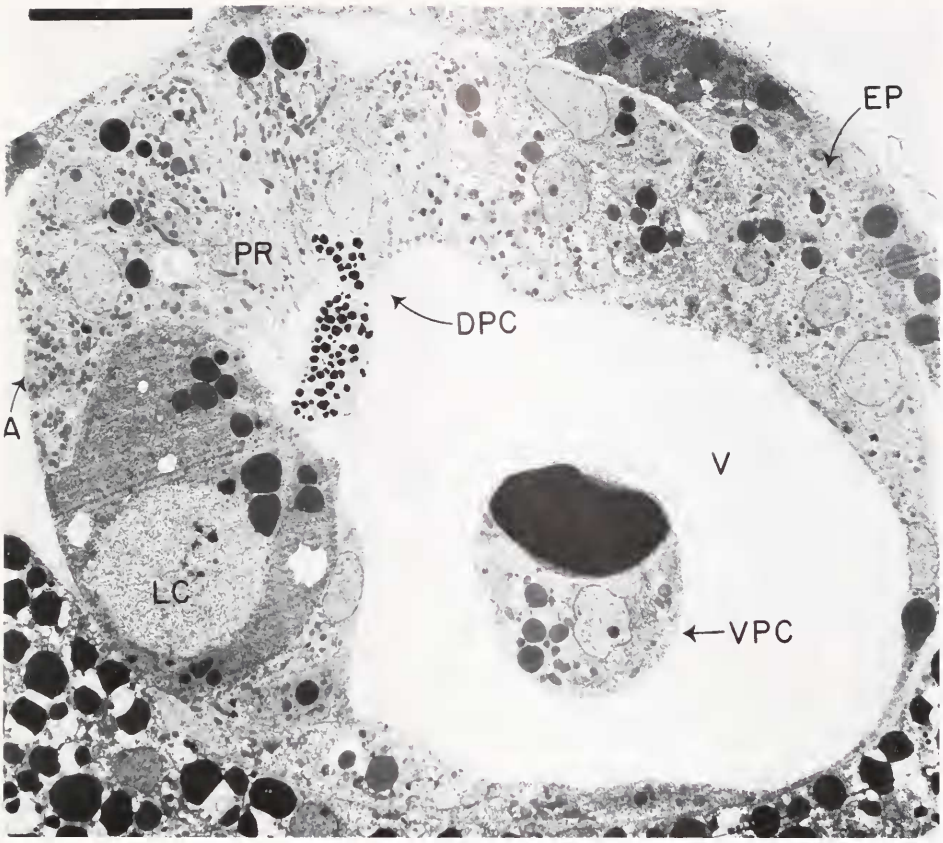

FIGURE 11. Electron micrograph of cross-section through the center of the prosencephalon. A = photoreceptor axons, DPC = dorsal melanocyte, EP = epidermis, LC = lens cell, PR = photoreceptor cells, V = ventricle, VPC = ventral melanocyte. Bar = 10 μm.

scriptions of the tadpole of the tunicate *Amaroucium constellatum*, and see Dilly and Wolken [1973] for a description of the ocellus of adult *Ciona*. Eakin [1973] and Salvini-Plawen and Mayr [1977] provide comprehensive general discussions of the evolution of visual organs.)

Yet another striking cell type characterizes the prosencephalon. A group of generally fusiform cells (4–5 μm wide) in the left ventro-caudal wall of the ventricle protrude tubulated bulbs (1–2 μm in diameter) into the ventricular lumen (Fig. 12). Each bulb sits on a ciliated stalk (9 + 0), and the axoneme runs up one edge of the bulb just inside the cell membrane. The remainder of the bulb is packed with generally parallel arrays of short membranous tubules with a fairly uniform diameter of 0.15–0.25 μm. The cytoplasm of the cell bodies is slightly more granular than the cytoplasm of the surrounding cells, and there also appear to be more mitochondria. Eakin and Kuda (1971), who report that occasional tubules of the bulbs open into the ventricular lumen, provide further ultrastructural descriptions of these unusual cells. A number of investigators (Dilly, 1969b; Eakin and Kuda, 1971; Olsson, 1975) have pointed out the remarkable structural similarities between the tubulated bulb cells of the tunicate CNS and the coronet cells of the teleost CNS. (See Jansen and Flight [1969], Harrach [1970], Galer and Billenstein [1972], and Rossi and Palombi [1976], for descriptions of the coronet cells.)

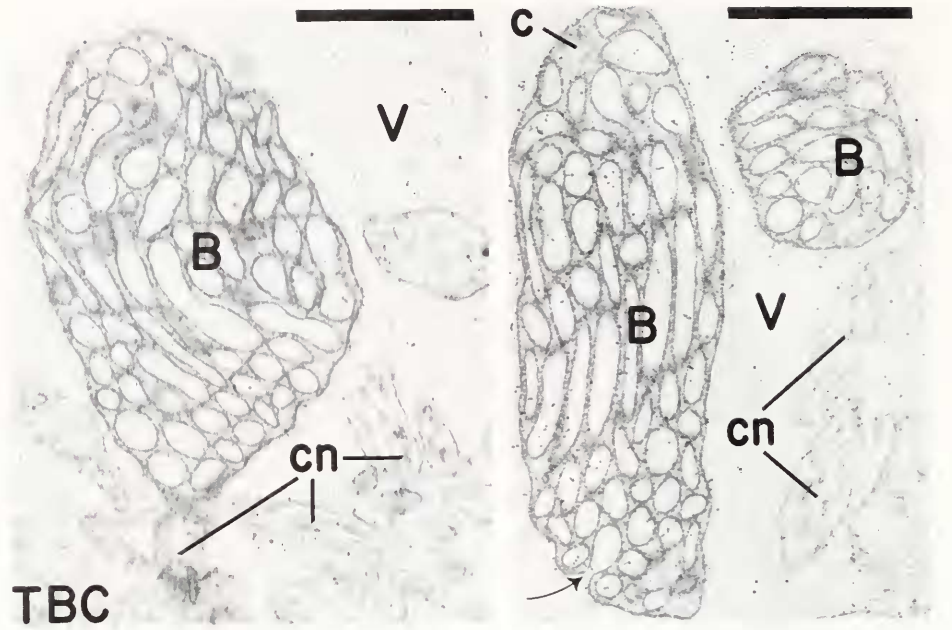

FIGURE 12. Electron micrographs of tubulated bulbs in the ventro-lateral prosencephalon. Arrow points to a natural opening between a membranous tubule of a bulb and the ventricle. B = tubulated bulb, c = "cilium" or axoneme of tubulated bulb, cn = ciliated necks of various tubulated bulb cells, TBC = tubulated bulb cell, V = ventricle of prosencephalon. Bar = 1 μm.

The prosencephalon contains two major axon tracts. On the right side, one axon tract of approximately 20 axons arises dorso-caudally from the bases of the photoreceptor cells and then courses caudally and ventrally as a flattened bundle of axons along the rim of the CNS (Fig. 11). The axons are 0.7–1.2 μm in diameter, and they run just inside the basal lamina, often in grooves of the endfeet of certain CNS cells. On the left side, another major axon tract (also approximately 20 axons) appears to arise ventrally from the vicinity of the tubulated bulb cells. These axons are 0.8–1.5 μm in diameter. They collect ventrally just inside the basal lamina of the CNS and then course caudally as a distinct bundle (still inside the basal lamina) along the ventral rim of the CNS. Both major prosencephalon tracts appear to contribute to the median basal axon tracts that form in the transitional CNS, between the prosencephalon and the deuterencephalon. This impression must still be confirmed by more detailed axon tracing techniques. In the prosencephalon, it is rare for any axons to be completely wrapped by a cell process of a surrounding cell, and there are no myelinated axons anywhere in the CNS of the *Ciona* tadpole. Myelination is a vertebrate characteristic (Peters *et al.*, 1976): truly myelinated axons probably do not exist in invertebrates (Bullock and Horridge, 1965; Bunge, 1970; Gutherie, 1975). Interestingly, lampreys, the most primitive extant vertebrates, also have no myelinated axons (Rovainen, 1979).

b. Deuterencephalon

Caudal to the prosencephalon, the CNS goes through a transitional zone. This region of the 19–20 h tadpole has a dynamic appearance—it is filled with growing cell processes (presumably axons), and it contains much extracellular space. The

transitional zone bulges to the right side of the animal behind the photoreceptors and the lens cells, and the deuterencephalon emerges caudally from the left half of the transitional zone.

The deuterencephalon begins in the caudal body at the head of the notochord and extends the length of the tail. The deuterencephalon lies directly along the dorsal surface of the notochord (Fig. 10). Just as with the prosencephalon, the deuterencephalon is a tube that is either one or two cells thick and that is completely surrounded by a fairly uniform basal lamina. Rostrally, the deuterencephalon is greater than 18 μm wide and 25 μm high; caudally, it tapers to less than 4 μm wide and 6 μm high.

A characteristic of the deuterencephalon at almost all levels is that its lumen is lined by four ciliated ependymo-glial cells, and four rows of these nonneuronal cells run in single files along the length of the caudal CNS. These cells appear to each have at least one 9 + 2 cilium, which can be more than 10 μm long.

Dorsally, a capstone ependymo-glial cell constitutes the entire dorsal wall of the deuterencephalon. This cell is wedge-shaped in cross-section and fusiform longitudinally. The capstone cell has "loose" vacuolated cytoplasm. Its nucleus is spherical or oblong (3 μm in cross-sectional diameter) with coarsely granular nucleoplasm.

Laterally, each ependymo-glial cell is crescent shaped in cross-section and fusiform longitudinally. Its nucleus is spherical (3–3.5 μm in diameter), with relatively dark and finely grained nucleoplasm. The ventral half of the basal surfaces of the lateral cells border the dorsal muscle cells. In the rostral deuterencephalon, the ventral surfaces of the lateral ependymo-glial cells border the two ventral deuterencephalic neurons. In the caudal deuterencephalon, axons of the lateral basal tracts run inside the basal lamina in grooves along the ventro-lateral surfaces of the lateral ependymo-glial cells.

Ventrally, the floor of the deuterencephalic ventricle is formed by a cuboidal cell. The floor cell has an oblong nucleus (1.5 $\times$ 4 μm cross-sectionally), and its cytoplasm contains many mitochondria and much dilated RER. In the rostral deuterencephalon, each floor cell lies above the median basal axon tract(s). In the caudal deuterencephalon, each floor cell extends to the ventral basal lamina of the CNS, thereby constituting the entire ventral wall of the deuterencephalon.

In the rostral deuterencephalon, a median basal axon tract (or perhaps a pair of adjacent axon tracts) runs between the ventral surface of the floor cell and the ventral basal lamina of the CNS (Fig. 13). The median basal tract(s) contains approximately 20–25 axons, ranging in diameter from 0.5 μm to 1.0 μm. These axons have descended from the prosencephalon and the transitional zone between the prosencephalon and the deuterencephalon. Some axons of the median basal tract(s) synapse in the rostral deuterencephalon. These synapses appear to include axo-somatic synapses (Fig. 13)—a characteristic of vertebrate nervous systems (Bullock and Horridge, 1965; Cohen, 1970; Bullock, 1974; Gutherie, 1975—Dilly [1975] reports finding axo-somatic synapses in one group of hemichordates but not in another [Dilly *et al.*, 1970]). The median basal tract(s) ends in the rostral tail.

In the caudal deuterencephalon, a lateral basal axon tract runs between the ventrolateral surfaces of the lateral ependymo-glial cell and the basal lamina, on each side of the CNS. Each of these tracts contains approximately 8–10 axons, ranging in diameter from 0.5–1.8 μm (Fig. 14). Axons of the lateral basal tracts make synapses with the dorsal muscle cells in the tail (Fig. 14). (See Flood [1975a], Cavey and Cloney [1976], and Mackie and Bone [1976] for descriptions of nerve-muscle junctions in various tunicates.) In addition to the lateral basal tracts, two axons (0.4–0.5 μm in diameter) run dorsally on each side of the caudal diencephalon in ventral grooves of the epidermal cells *outside* the basal lamina of the CNS. The

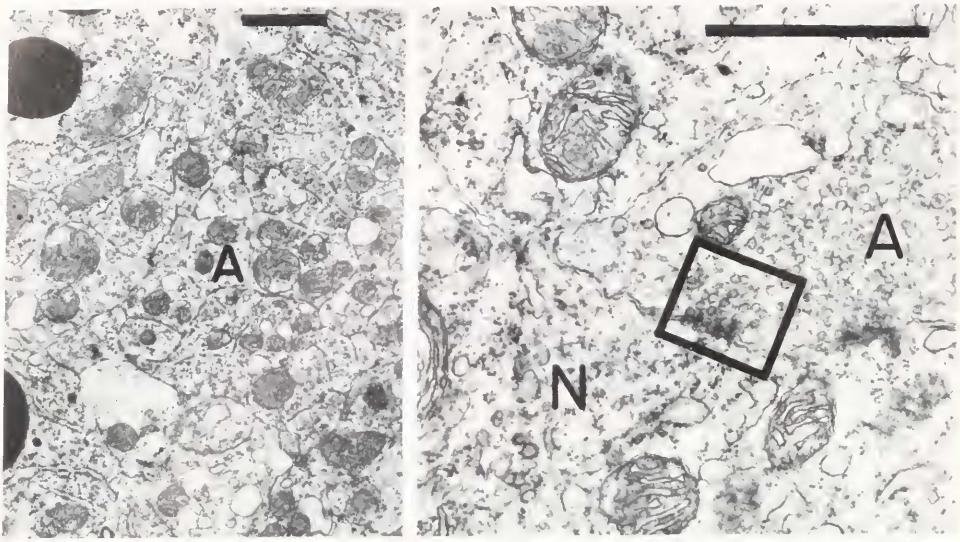

FIGURE 13. Electron micrographs from the rostral deuterencephalon. Left: median basal axon tract(s). Right: axo-somatic synapse (in box). A = axon, N = neuron cell body. Bar = 1 μm.

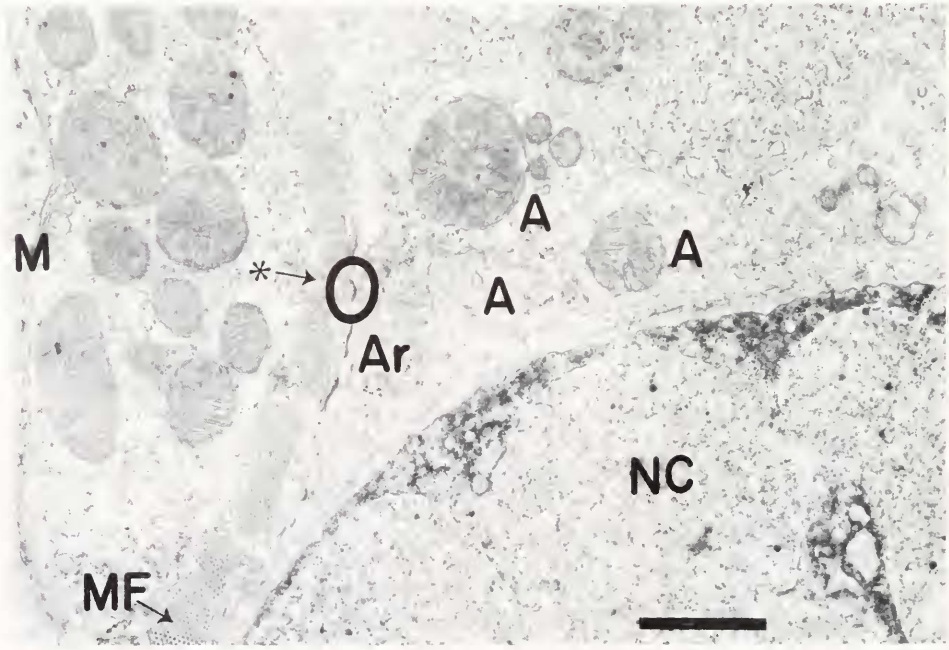

FIGURE 14. Electron micrograph of nerve-muscle junctions in the tadpole tail. Axons are in the lateral basal tract of the deuterencephalon. Asterisk and circle indicate a junction between an axonal rootlet and a dorsal muscle cell; vesicles are accumulated in the pre-synaptic area, and there is a post-synaptic thickening. A = axon, AR = axonal rootlet, M = muscle cell, MF = myofibril, NC = notochord cell. Bar = 1 μm.

exact courses, the origins, and the terminations of these axons as well as further details of the median and the lateral basal tracts must be determined by more specific axon tracing studies. Likewise, specific staining studies must still be done to definitively identify the locations of all CNS neurons.

The general organization of the tail deuterencephalon of *Ciona* tadpoles is quite similar to that of other ascidian tadpoles. Bone and Mackie (1976) found that in *Dendrodoa* a set of ciliated ependymo-glial cells surround the ventricular lumen, and two lateral basal tracts of 5–6 axons each run in grooves along the ventrolateral edges of the CNS. *Dendrodoa* also has a bundle of about 4 axons running through the tail outside the CNS along a groove in the base of the epidermal cells. In contrast to *Ciona, Dendrodoa* tadpoles have a pair of axons running longitudinally (outside the CNS) along the most ventral muscle cells beside the anterior segment of the endodermal strand. Torrence and Cloney (1982) have described a very similar organization in *Diplosoma. Diplosoma* tadpoles do not appear to have a pair of axons running between the ventral muscle cells and the endodermal strand, but they do have a bundle of about 50–70 ventral axons running below the endodermal strand, along a groove in the base of the epidermal cells, in a sort of mirror-image of similar dorsal bundles of axons above the deuterencephalon. These mirror-image dorsal and ventral axon bundles arise from pairs of ciliated sensory cells embedded in the tail epidermis along the midline.

Organ system rudiments

1. Primordial pharynx

A one cell-thick pocket of cuboidal and columnar cells forms the primordial pharynx in the dorsal epidermis of the tadpole body. The primordial pharynx is an oblong tubular body capping the rostral end of the endodermal cavity and then extending caudally over the rostral tip of the CNS (Figs. 2, 4). The external opening of the lumen of the primordial pharynx is directly over the CNS. Ventrally, the ventricle of the CNS prosencephalon extends as a small bore (3–4 μm diameter) ciliated tube under the primordial pharynx. The most rostral end (the 'neuropore') of this ventricle opens into the rostral end of the lumen of the primordial pharynx.

The cells of the primordial pharynx are about 6 μm wide and 9 μm long. Their cytoplasm is slightly darker and has a more finely grained texture than that of the surrounding epidermal cells. The primordial pharyngeal cells are filled with RER in all states of dilation. Nuclei are spherical (3–4 μm in diameter). The luminal surfaces of the primordial pharyngeal cells are fairly smooth and are covered with forming coated pits. In the region of the neuropore, the floor of the primordial pharynx is formed by cells of the prosencephalon. These cells protrude microvilli and cilia into the rostral lumen of the primordial pharynx.

2. Atrial primordia

Pockets of similar cuboidal and columnar cells also form the two atrial primordia, one on the left and one on the right, in the epidermis of the body wall (Figs. 4, 10, 15). The atrial primordia border the gut primordium at a level just rostral to the head of the notochord. Each atrial primordum is a spherical pocket with walls which are one cell thick; at its widest cross-section (28–30 μm diameter), an atrial primordium consists of five or six cells (Figs. 10, 15). Each atrial cell is about 8–10 μm wide and 13–15 μm long. Its cytoplasm has dilated RER. Nuclei are oblong or spherical (3–5 μm in diameter). The luminal surfaces of the cells of the atrial pri-

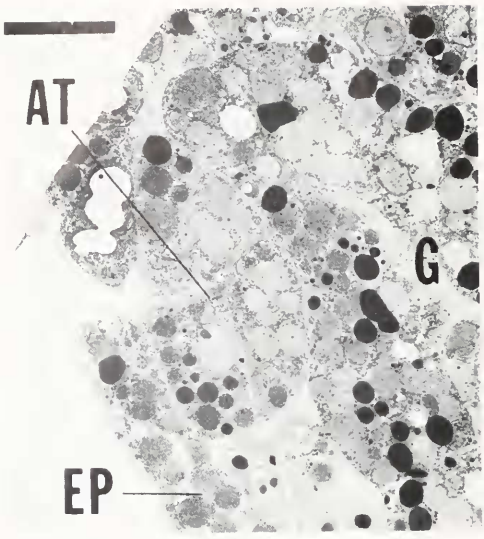

FIGURE 15. Electron micrograph of an atrial primordium in the lateral wall of the tadpole body. AT = atrial primordium, EP = epidermis, G = gut primordium. Bar = 10 μm.

mordia are smooth. During metamorphosis, the two atrial primordia fuse and form the single atrial siphon of the adult (Berrill, 1950). For a general description of the development of the atria, see Willey (1893a).

3. Gut primordium

In the 19–20 h tadpole, the gut primordium is a closed folded tube, one cell thick, that fills the ventral two-thirds of the body (Figs. 2, 4, 15, 16). The gut lumen (the 'endodermal cavity') extends from the pharyngeal primordium rostrally to the head of the notochord caudally. The gut primordium is followed in the tail by the 'endodermal strand'—a lumenless set of gut cells, in single file, lying directly under the notochord along its entire length (Figs. 2, 7, 8).

The prosencephalon lies directly along the top wall of the gut primordium. In the rostral half of the tadpole body, the bottom wall of the gut primordium lies directly along the ventral epidermis. The rostral end of the gut primordium borders an extracellular space behind the adhesive organ. This space is called the 'pre-oral lobe'; it contains separated spherical cells and will apparently continue to fill with cells before metamorphosis (Willey, 1893a).

Cells of the gut primordium and of the endodermal strand are roughly cuboidal (4–8 μm wide) and are characterized by large numbers of yolk vesicles. The cytoplasm of these cells appears "loose" and vacuolated and contains few organelles. At 19–20 h, the luminal surfaces of the gut cells are irregular but have neither cilia nor extensive microvilli. At this developmental stage, there do not appear to be any specialized gland cells.

4. Mesodermal pockets

The 19–20 h *Ciona* tadpole contains a number of pockets of mesodermal cells (Conklin, 1905). Four prominent pockets (two bilateral pairs) are found between the ventrolateral walls of the gut primordium and the ventrolateral epidermis (Figs.

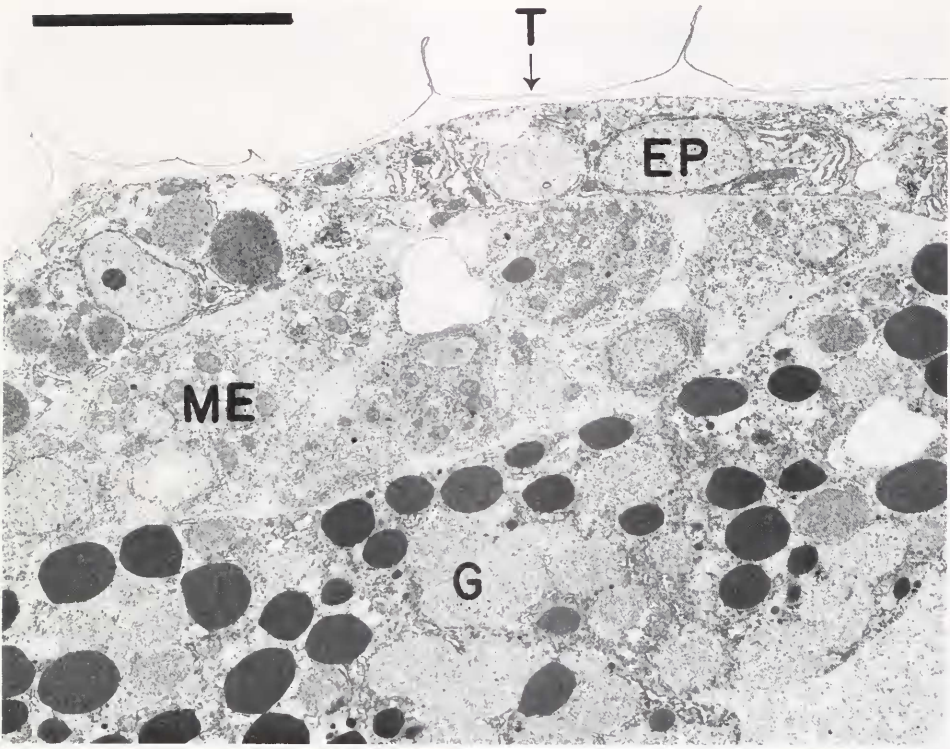

FIGURE 16. Mesodermal pocket in the ventro-lateral body wall of the tadpole. EP = epidermis, G = gut primordium, ME = mesodermal pocket cell, T = tunic. Bar = 10 μm.

2, 4, 16). The mesodermal cells are approximately spherical (5–6 μm in diameter) and are filled with strikingly dilated RER (Fig. 16). The numerous mitochondria appear to be slightly larger (up to 1 μm in cross-sectional diameter) than those of surrounding cells, and the cytoplasm of the mesodermal cells is less granular and contains fewer yolk vesicles than the surrounding cells. Mesodermal cell nuclei are roughly spherical (3 μm in diameter). Presumably, many of these cells will give rise to the musculature of the adult organism.

DISCUSSION

The first important conclusion from these observations is that the gross anatomy in the old descriptions of tunicate tadpoles can for the most part still be accepted today. On the other hand, the fine histological details were often inaccurate in the old reports, and modern ultrastructural analyses must form the bases for anatomical descriptions at the cellular level.

Basically, the tunicate tadpole is an extremely simple organism. In *Ciona* tadpoles, there appear to be fewer than 20 morphologically distinct cell types, which are organized into 6 clearly distinct and well developed organ systems and into about 4 organ system rudiments. Of the developed organ systems, the nervous system—which includes neurons, ependymo-glial cells, pigment cells, and at least two distinct types of receptor cells—contains the greatest variety of cell types. Besides its small number of cell types, the *Ciona* tadpole is also remarkable in the small

total numbers of cells that compose its organs. For example, the central nervous system appears to contain fewer than 50 neurons, and this means that the *Ciona* tadpole has what may be the simplest nervous system constructed in the general vertebrate plan.

Vertebrate homologies

Anatomical descriptions of the tunicate tadpole are important to biologists because of the position of the tunicates in chordate phylogeny. It is generally believed that the tunicates of today are descendants of a form of animal that was transitional between the deuterostomian invertebrates (notably, the echinoderms) and the vertebrates (Brooks, 1893; Garstang, 1894, 1928; Van Name, 1921; Berrill, 1955; Romer, 1970; Plough, 1978; Jefferies, 1979). From various biochemical comparisons, Ohno (Atkin and Ohno, 1967; Ohno, 1970; Fisher *et al.*, 1980) has suggested that major gene duplications (either polyploidisations or regional duplications) were instrumental in the divergence of the other chordates from an ancestral tunicate-like stock.

To draw evolutionary inferences from comparative anatomical data, the compared anatomies must be homologous. Homologous anatomies develop from genomic sequences that were identical in an ancestral stock of organisms (De Beer, 1958). Since Kowalevsky first proposed the idea in 1866, many comparative anatomists have considered the body plans of the tunicate tadpole and the vertebrate embryo to be homologues. One strong argument for this homology includes a list of the many sequentially and spatially distinct developmental steps that are apparently shared by the tunicates and the vertebrates. Here, the underlying assumption is that each sequentially or spatially distinct developmental step probably involves at least one different genomic sequence. Thus, many shared sequentially or spatially distinct developmental steps imply a significant number of shared genomic sequences.

As with all deuterostomes (Meglitch, 1972), tunicate embryogenesis begins with a series of fairly straightforward orthogonal cleavages (Conklin, 1905). Later, the blastopore becomes the caudal end of the trunk, and the "mouth" (in the tunicate, the primordial pharynx) develops secondarily at the opposite end of the embryo (Sedgewick, 1889; Willey, 1893a, b; Conklin, 1905). In tunicates, as in all of the vertebrates, the neural tube rolls up dorsally from a dorsal neural plate, the tail develops as a dorsal extension beyond the caudal end of the trunk, the gut develops ventrally, a notochord develops centrally and underlies the entire caudal neural tube, and muscles develop bilaterally flanking the notochord (Kowalevsky, 1866; Willey, 1893a, b; Conklin, 1905; MacBride, 1914; Satoh, 1978). These developmental steps give rise to an organism with a basic vertebrate topology, as diagrammed in Fig. 17. It is intriguing that incomplete gastrulation can lead to a larval vertebrate with a body plan that is even more strikingly like a tunicate tadpole—see figures 6 and 7 in Moore (1946). (In addition, the tunicate endostyle may be homologous to the vertebrate thyroid gland [Brooks, 1893; Barrington, 1957; Thorpe and Thorndyke, 1975].)

Vertebrate embryos typically develop two additional tubular systems (Goodrich, 1930; Neal and Rand, 1936; Ballard, 1964; Poole and Steinberg, 1981). Centrally, between the notochord and the gut, a major artery (or a pair of major arteries) extends the length of the caudal half of the animal. Furthermore, bilateral excretory ducts of the genito-urinary system run adjacent to the arteries through the vertebrate trunk. *Ciona* tadpoles do not appear to develop distinct homologues to these basic

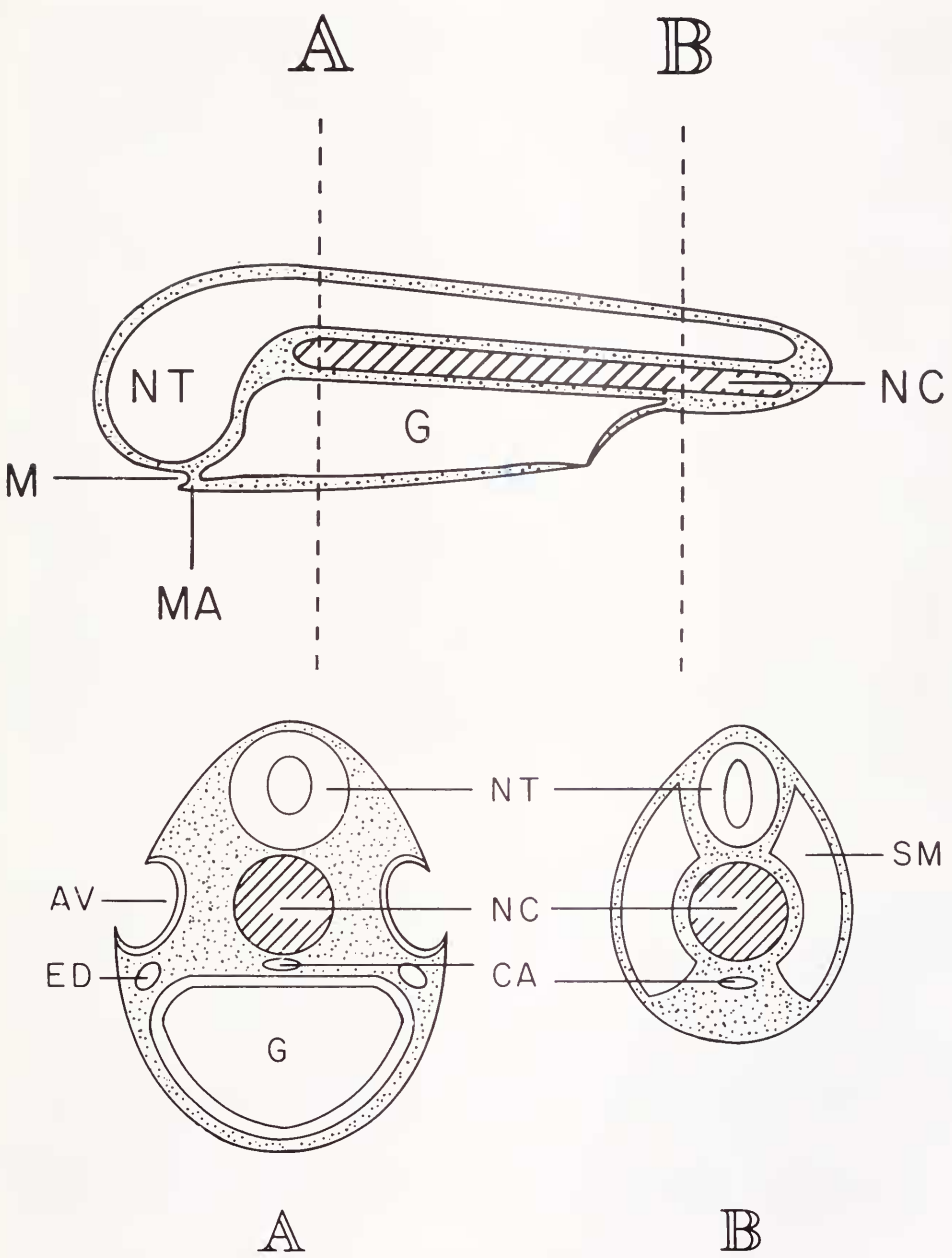

FIGURE 17. a: Schematic sagittal section of a characteristic vertebrate embryo. b: Schematic cross-sections at the two levels indicated in figure 17a. AV = auditory vesicle, CA = central artery, ED = genito-urinary excretory duct, G = gut primordium, M = mouth, MA = mandibular arch, NC = notochord, NT = neural tube, SM = somatic musculature.

vertebrate tissues. On the other hand, these tissues are largely mesodermal in origin, and the bilateral pockets of mesoderm in the tunicate tadpole may be related to the precursor tissues of the vertebrate circulatory and genito-urinary systems. (The tu-

nicate heart does develop from a ventral pocket of mesoderm cells, but in the 19–20 h *Ciona* tadpole this organ has not yet formed [Selys-Longchamps, 1900].)

If the tunicate tadpole and the vertebrate embryo do in fact have essentially homologous topologies, then two other anatomical homologues can be suggested. First, all vertebrates develop auditory vesicles from bilateral invaginations of the body ectoderm at about the level of the head of the notochord (McEwen, 1931; Yntema, 1055; Ballard, 1964; Thornhill, 1972; Jarvik, 1980; Model *et al.*, 1981). The extinct mitrates, probable precursor organisms to all the chordates, also appear to have had homologous bilateral vesicles, called 'atria' (Jefferies and Lewis, 1978; Jefferies, 1981). Moreover, Bone and Ryan (1978) have shown that the atrium of the adult tunicate develops ciliated sensory cells in cupular organs resembling those of the typical vertebrate acoustico-lateralis system. Thus, the bilateral atrial primordia of the tunicate tadpole may be homologous to the inner ear primordia of the vertebrates. (Bone and Ryan [1978] have suggested essentially the same homology.)

Second, vertebrate embryos develop bilateral bulges, the embryonic mandibular arches, just ventral to the mouth (McEwen, 1931; see Neal and Rand, 1936, Bjerring, 1977, and Jarvik, 1980, for discussions of the terminology and the anatomy of the embryonic mandibular arch region, which appears to develop two distinct somites—a premandibular somite and a mandibular somite—in many vertebrates). In amphibians, transient adhesive organs develop at the ends of these bulges (Harrison, 1925; McEwen, 1931; Schotte and Edds, 1940; Eakin, 1963; Perry and Waddington, 1966; Lyeral and Pelizzari, 1973; Roberts and Blight, 1975). There may also be homologous adhesive organs in the larvae of teleost fishes (Kerr, 1919). (But see Garstang [1928] for an alternative discussion.) Might the adhesive organs and their foundation tissues in the tunicate tadpole be homologous with the embryonic mandibular arches of the vertebrates?

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